PROTEINS OF RAT BRAIN MYELIN. EXTRACTION WITH SODIUM DODECYLSULPHATE AND ELECTROPHORESIS ON ANALYTICAL AND PREPARATIVE SCALE

T.V.WAEHNELDT* and P.MANDEL
Centre de Neurochimie du CNRS, 11 rue Humann, 67-Strasbourg, France

Received 2 July 1970

1. Introduction

In contrast to the lipid components of myelin, relatively little research has been done so far on the protein portion, due to the difficult task of gently extracting myelin proteins from their lipid matrices. Most of the procedures involve extraction of part of myelin proteins, either with chloroform-methanol mixtures [1] or with dilute acids [2]. Only recently, the extraction of total protein of myelin with a mixture of phenol-formic acid-water and subsequent electrophoretical analysis in the same system has been reported [3, 4].

In this paper we describe 1) how total protein of rat brain myelin is extracted by the use of sodium dodecylsulphate (SDS) and 2) how these extracts are examined by electrophoresis on analytical polyacrylamide gels giving three predominant bands. We also show that this method, developed by E.M.Shooter and his associates [5], can be transferred to a preparative scale, thus allowing the quantitative separation of all protein components of myelin.

2. Materials and methods

Brains of mature rats were homogenized in 0.32 M sucrose, the resulting suspension was centrifuged at 5,000 rpm for 20 min in rotor R30 (Beckman Instruments). The sediment was washed twice with 0.32 M sucrose under identical conditions of centrifugation.

* Chargé de Recherche au CNRS.

The pellet finally obtained was homogenized in 0.88 M sucrose and centrifuged at 25,000 rpm for 2 hr in the SW 25.1 rotor (Beckman Instruments). Material floating on 0.88 M sucrose was subjected to osmotic shock, then washed three times at low speed with water (R30, 5,000 rpm, 20 min), followed by a second high-speed centrifugation in 0.88 M sucrose. This process of three-fold washing and single high-speed distribution was repeated twice, first with 0.80 M sucrose, then with 0.75 M sucrose. Material forming a pellet during the second 0.88 M sucrose step was called A, material floating on 0.88 M, 0.80 M or 0.75 M sucrose was called B, C, or D, respectively.

Extraction of proteins from myelin fractions A, B, C, and D was performed by homogenization in 0.1% SDS-0.01 M tris (pH 7.4); separation from traces of insoluble material was done by centrifugation at 40,000 rpm for 2 hr in the R40 rotor (Beckman Instruments).

For analytical polyacrylamide gels the following solutions were prepared:

for the resolving gel:

I) 12 g acrylamide, 0.32 g *N,N'*-methylenebisacrylamide (BIS), 6 ml 1 N HCl, 4.75 g tris, 0.1 g SDS, 0.06 ml *N, N, N', N'*-tetramethylethylenediamine (TEMED), 3.75 mg potassium ferrocyanide, made up to a total of 50 ml with water.

II) 70 mg Ammonium persulphate in 50 ml water.

III) 6 ml 1 N HCl, 4.75 g tris, 0.06 ml TEMED, 0.1 g SDS in a total of 100 ml.

for the stacking gel:

IV) 2.5 g acrylamide, 0.6 g BIS, 18 g sucrose, 3.07

ml 1 M phosphoric acid, 0.68 g tris, 0.01 ml TEMED, 0.1 g SDS, 0.5 mg riboflavin in a total of 100 ml.

V) 3.07 ml 1 M phosphoric acid, 0.68 g tris, 0.01 ml TEMED, 0.1 g SDS in a total of 100 ml.

Tubes of 5 mm inner diameter and 130 mm length were filled with 1.2 ml of a mixture of equal volumes of solutions I and II. Solution III (0.2 ml), layered over the resolving gel solution, served for obtaining a horizontal surface. Solution III was then decanted and replaced with 0.2 ml of solution IV, over which 0.2 ml of solution V was carefully layered. After polymerization, the gels were loaded with 0.2–0.4 ml of SDS extracts of myelin (0.6–0.8 mg protein/ml). Composition of upper buffer: 6.32 g tris, 3.94 g glycine, 1.00 g SDS, in 1,000 ml. Lower buffer: 12.1 g tris, 50 ml 1 N HCl, 1.00 g SDS, in 1,000 ml. The initial current of 0.1 mA per tube was gradually increased to 1 mA per tube at the end of the run. Gels were stained with 0.1% amidoblack in 10% acetic acid and destained in 10% acetic acid.

The chemical composition of the preparative gel was identical to that of smaller-sized analytical gels. The inner diameter of the tube was 24 mm, the length of resolving gel was 105 mm, that of the stacking gel 10 mm. The column was loaded with 20 ml myelin extract (12–16 mg myelin protein). The current was gradually increased from 3 mA to 18 mA. Fractions were taken at 20 min intervals for analysis on analytical gels.

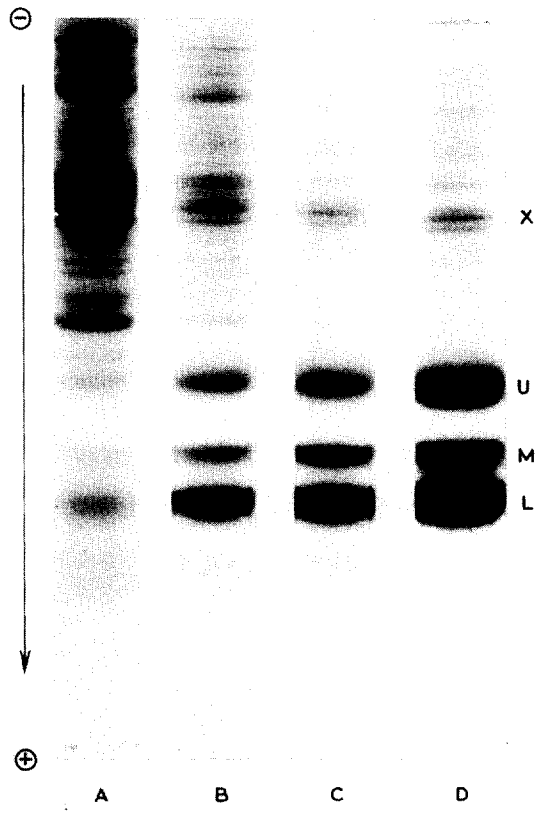

Fig. 1. Polyacrylamide gel electrophoresis of SDS extracts of rat brain myelin fractions. A, B, C, and D represent membrane fractions of increasing purity. 12% polyacrylamide, tris-glycinate system, pH 8.9, 0.1% SDS in samples, gels, and buffers.

3. Results and discussion

SDS extracts of myelin preparations A, B, C, and D originating from the last three phases of the isolation scheme were analyzed for proteins by electrophoresis on 12% polyacrylamide gels (fig. 1). The increase in purity of myelin from preparations A to D is reflected by an increase in three major bands in the lower part of the gels. These bands are called L, M, and U (for low, middle, and up). A rapid decrease from gels A to D of most of the numerous bands in the upper gel portion, with the exception of some minor bands at the location X, was also observed. The question whether the protein bands near X are representative of myelin or are residual impurities cannot yet be answered. Bands L, M, and U, however, are truly of myelin origin. Tracings of gel D give, upon integration, 93% for L + M + U. This result taken in conjuction with the known predominance in myelin of lipids over proteins indicates a rather pure myelin preparation.

In the SDS-polyacrylamide gel system, the molecular weight of proteins is reflected by the rate of migration [6]. Based on the results of Mehl and Halaris [4] who also observe three bands for rat brain myelin, we can assume that fraction U represents larger molecular weight proteolipid protein, whereas L and M are smaller molecular weight basic proteins.

Treatment of membranes with SDS generally leads to extraction of total proteins regardless of their

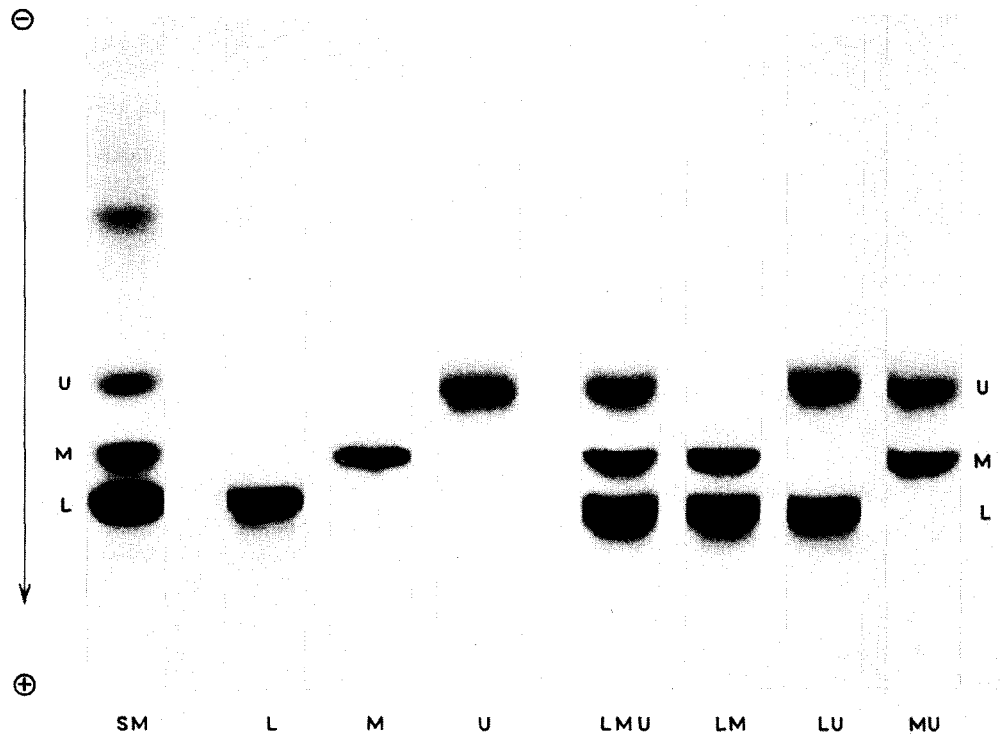

Fig. 2. Electrophoretical analysis of rat brain myelin proteins. Protein fractions L, M, and U were isolated from crude myelin by SDS extraction and subsequent separation on a preparative polyacrylamide gel column. For details see fig. 1.

charge and structural involvement with other membrane components [7]. Therefore, a procedure for the preparative separation of myelin proteins L, M, and U has been accomplished by enlarging the dimensions of the polyacrylamide gels applied. The results of this electrophoretic separation are shown in fig. 2. To the left is the electrophoresis of the SDS extract of the starting material (SM), in this case a crude myelin preparation. To the right are purified fractions, L, M, and U. Each fraction is essentially devoid of contamination by other fractions. Remixing fractions L, M, and U gives the corresponding composite gel patterns. Analysis of fractions L, M, and U for lipids is entirely negative; all lipids leave the polyacrylamide column with the front.

In summary: purification steps for the preparation of myelin can be followed by analytical gel electrophoresis of SDS extracts. Rat brain myelin gels show three predominant bands (L, M, U). These can be separated on a preparative scale*. Thus total protein of myelin isolated as pure protein-SDS complexes, can be studied [10].

References

[1] J.Folch and M.Lees, J. Biol. Chem. 191 (1951) 807.
J.Folch, in: Biochemistry of the Developing Nervous System, ed. H.Waelsch (Academic Press, New York, 1955) p. 121.
[2] G.E.Deibler, R.E.Martenson and M.W.Kies, Biochim. Biophys. Acta 200 (1970) 342.
R.E.Martenson, G.E.Deibler and M.W.Kies, Biochim. Biophys. Acta 200 (1970) 353.
[3] E.Mehl and W.Wolfgram, J. Neurochem. 16 (1969) 1091.
[4] E.Mehl and A.Halaris, J. Neurochem. 17 (1970) 659.
[5] R.M.Grossfeld, Thesis, Stanford University, 1968.

* A detailed account of the results described in this short communication is in preparation [8, 9].

T.V.Waehneldt, R.M.Grossfeld and E.M.Shooter, Abstr. Second Intern. Meeting Intern. Soc. Neurochemistry, Milan 1969, p. 411.
[6] A.L.Shapiro, E.Viñuela and J.V.Maizel, Biochem. Biophys. Res. Commun. 28 (1967) 815.
[7] T.V.Waehneldt and E.M.Shooter, in preparation.
[8] T.V.Waehneldt and P.Mandel, in preparation.
[9] T.V.Waehneldt, in preparation.
[10] J.P.Zanetta, T.V.Waehneldt, P.Mandel and G.Gombos, in preparation.